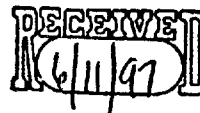




XetaPharm, Inc.
100 Jersey Avenue
Building B, Suite 310
New Brunswick, NJ 08901-5279
(908) 249-0133
Fax (908) 247-4090

June 3, 1997



Dr. Elizabeth A. Yetley, Director
Office for Special Nutritionals, HFS-450
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, N.W.
Washington, D.C. 20204

0175 '97 SEP 19 P1:48

Dear Dr. Yetley:

Notice is hereby given pursuant to the requirements of Section 403(r)(6)(21 U.S.C. 343 (r)(6)) of the Federal Food, Drug and Cosmetic Act of statements of nutritional support which have been made on the label and in the labeling in connection with the marketing of the dietary supplement *GinsengOnce™*, 600 mg tablet. *GinsengOnce™*, 600 mg tablet was first marketed with these statements of nutritional support on May 3, 1997. The statements of nutritional support are as follows:

Carton Label: Front

GinsengOnce™
Once-Per-Day Herbal Dietary Supplement

Only One Tablet Per Day
600 mg Ginseng Extract

Unique Dual-Release Tablet

One 300 mg Layer Releases Slowly Over Several Hours
One 300 mg Layer Releases Quickly

Provides Nutritional Support During Periods of Stress or Fatigue*

30 Day Supply
30 Bi-Layered Tablets, 600 mg Each

975-0162

53009
LET 681



Dr. Elizabeth A. Yetley, Director
Office of Special Nutritionals
Page 2 , June 3, 1997

Carton Label: Back

Our **Unique Dual-Release Tablet** releases one 300 mg layer of ginseng extract quickly for immediate support, and one 300 mg layer slowly over several hours for sustained support throughout the day.

The purity and potency of *GinsengOnce™* has been verified by High Performance Liquid Chromatography (HPLC).

Recommendations: Take one tablet daily at mealtime.

Ginseng has been traditionally used for its ability to increase the body's resistance to stress and fatigue.*

*THESE STATEMENTS HAVE NOT BEEN EVALUATED BY THE FOOD AND DRUG ADMINISTRATION. THIS PRODUCT IS NOT INTENDED TO DIAGNOSE, TREAT, CURE, OR PREVENT ANY DISEASE.

CAUTIONS: DO NOT USE IF YOU ARE PREGNANT, LACTATING OR HAVE HIGH BLOOD PRESSURE AS WITH ANY SUPPLEMENT, CONSULT YOUR HEALTHCARE PRACTITIONER BEFORE USING THIS PRODUCT.

KEEP OUT OF REACH OF CHILDREN

A Carton is attached.

Labeling:

Front Page: A High-Potency Ginseng Product
 In a Convenient Once-Per-Day Dosage

Announcing *GinsengOnce™*

Features

- Once-Per-Day
- 60 mg Ginsenosides
- Dual-Release Tablet

Unique Dual-Release Tablet

One 300 mg Layer Releases Slowly Over Several Hours
One 300 mg Layer Releases Quickly

Back Page:

GinsengOnce™
Offers Convenience, Quality and Value

Convenience

- *GinsengOnce™* provides 600 mg of standardized *Panax ginseng* extract in **a convenient Once-Per-Day dosage**. The Unique Dual-Released Tablet releases one 300 mg layer of ginseng extract quickly for immediate support, and one 300 mg layer slowly over several hours.

Quality

- *GinsengOnce™* contains *Panax ginseng* root extract standardized to **10% ginsenosides**.
- **The purity and potency of *GinsengOnce™* is laboratory verified** by one of the most accurate analytical methods available, High Performance Liquid Chromatography (HPLC).
- **Dissolution tests are conducted on *GinsengOnce™*** to ensure the product dissolves according to its design. The results are verified by HPLC.

Value

- *GinsengOnce™* is a better value than other leading brands. *GinsengOnce™* **costs less than 60 cents a day** at the Suggested Retail Price of \$17.95 per 30 Tablet Carton.

Dr. Elizabeth A. Yetley, Director
Office of Special Nutritionals
Page 4 , June 3, 1997

Back Page: Side Panel

Product Features

- Once-Per-Day
- 600 mg Extract
- Dual-Release Tablet
- 60 mg Ginsenosides
- Laboratory Tested
- Costs Less than 60 Cents a Day

XetaPharm™
100 Jersey Avenue
Building B, Ste. 310
New Brunswick, NJ 08901-3279
Tel: (908) 249-0133
Fax: (908) 247-4090

A Labeling copy is attached.

A typical High Performance Liquid Chromatogram of *GinsengOnce™* is attached.

Very truly yours,
XetaPharm, Inc.
A subsidiary of
Xechem, International Inc.



Ramesh Chandra Pandey, Ph.D.
President and CEO

Ginseng Once

Once-Per-Day Herbal Dietary Supplement

30 Bi-Layered Tablets, 600 mg Each

Ginseng Once

Once-Per-Day Herbal Dietary Supplement

Only
One Tablet
Per Day

600 mg Ginseng Extract

Unique
Dual-Release Tablet



Provides Nutritional Support During
Periods of Stress or Fatigue

30 Day Supply

30 Bi-Layered Tablets, 600 mg Each

Once-Per-Day Herbal Dietary Supplement 30 Bi-Layered Tablets, 600 mg Each

Ginseng Once

Our Unique Dual-Release Tablet releases one 300 mg layer of ginseng extract quickly for immediate support, and one 300 mg layer slowly over several hours for sustained support throughout the day.

The purity and potency of *GinsengOnce*™ has been verified by High Performance Liquid Chromatography (HPLC).

Recommendations: Take one tablet daily at mealtime.

Ginseng has been traditionally used for its ability to increase the body's resistance to stress and fatigue.*

*These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

CAUTIONS: DO NOT USE IF YOU ARE PREGNANT, LACTATING OR HAVE HIGH BLOOD PRESSURE. AS WITH ANY SUPPLEMENT, CONSULT YOUR HEALTHCARE PRACTITIONER BEFORE USING THIS PRODUCT.

KEEP OUT OF REACH OF CHILDREN

Supplement Facts

Serving Size; 1 Tablet

	Amount Per Tablet
<i>Panax ginseng</i> Root Extract	600 mg*
Ginsenosides(10%)	60 mg*

* Daily Value Not Established

Other Ingredients: Dicalcium Phosphate, Hydroxypropylmethylcellulose, Microcrystalline Cellulose, Maltodextrin, Stearic Acid, Cottonseed Oil, Silica, Magnesium Stearate, Croscarmellose Sodium, Wheat Germ Powder, Pharmaceutical Glaze and Riboflavin.

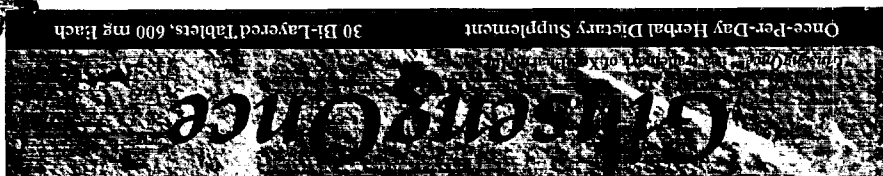
Distributed by:
XetaPharm, Inc., New Brunswick, NJ 08901 USA

Free From: Artificial colors, preservatives, flavors, soy, yeast, dairy products, animal or fish derivatives.

Store in a cool, dry room; 59°-86°F (15°-30°C).

Each tablet is individually safety-sealed for your protection. Do not use if the seal is broken.

PROD#: P115 Lot#



6
01/97

**A High-Potency Ginseng Product
in a Convenient Once-Per-Day Dosage.**

Announcing

GinsengOnce™



Features

- **Once-Per-Day**
- **60 mg Ginsenosides**
- **Dual-Release Tablet**

One 300 mg Layer
Releases Slowly
Over Several Hours

**Unique
Dual-Release Tablet**



One
300 mg Layer
Releases Quickly

Product Features

- Once-Per-Day
- 600 mg Extract
- Dual-Release Tablet
- 60mg Ginsenosides
- Laboratory Tested
- Costs Less Than 60 Cents a Day

GinsengOnce™

Offers Convenience,
Quality and Value

Convenience

- *GinsengOnce*™ provides 600 mg of standardized *Panax ginseng* extract in a **convenient Once-Per-Day dosage**. The Unique Dual-Released Tablet releases one 300 mg layer of ginseng extract quickly for immediate support, and one 300 mg layer slowly over several hours.

Quality

- *GinsengOnce*™ contains *Panax ginseng* root extract standardized to **10% ginsenosides**.
- **The purity and potency of *GinsengOnce*™ is laboratory verified** by one of the most accurate analytical methods available, High Performance Liquid Chromatography (HPLC).
- **Dissolution tests are conducted** on *GinsengOnce*™ to ensure the product dissolves according to its design. The results are verified by HPLC.

Value

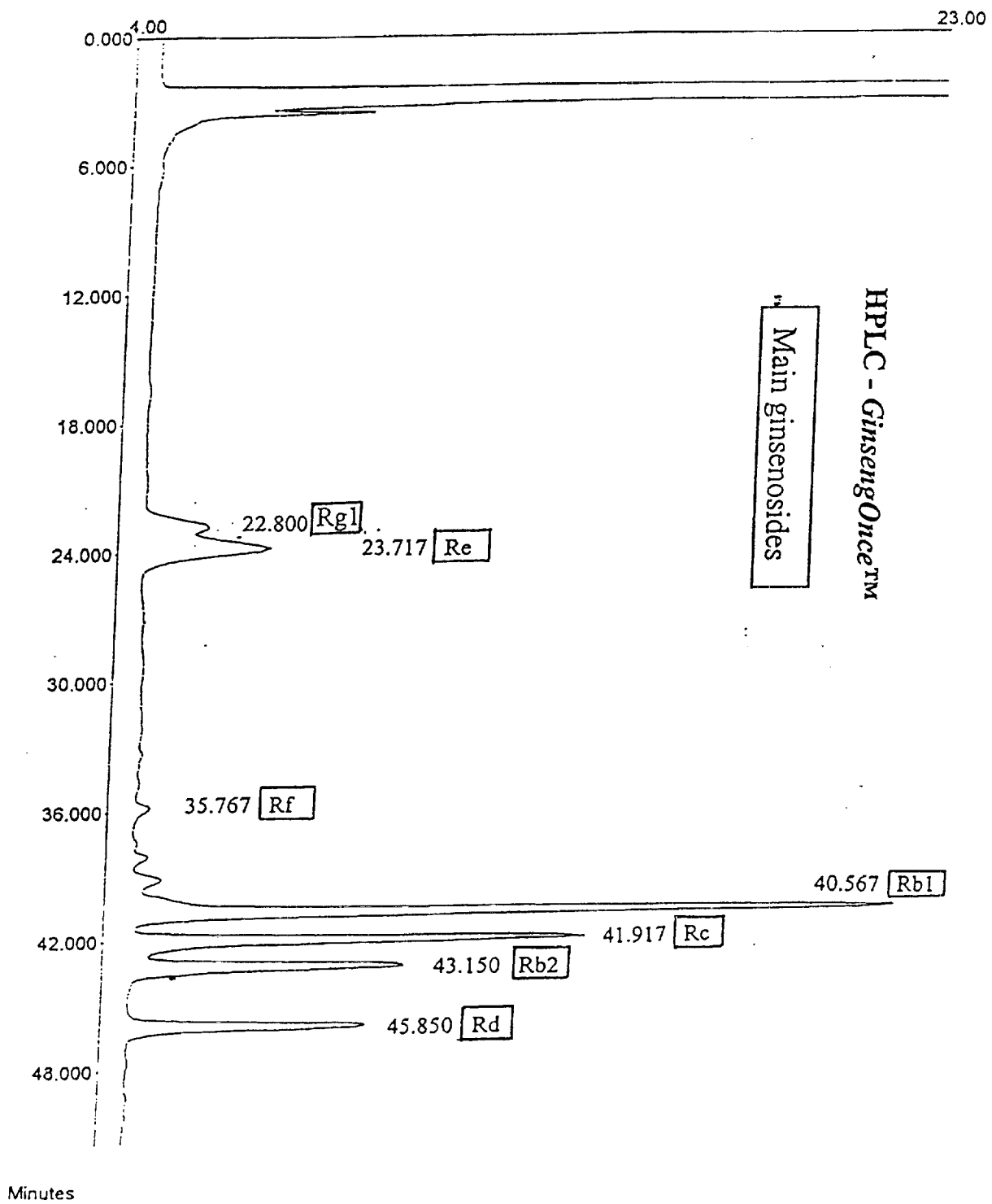
- *GinsengOnce*™ is a better value than other leading brands. *GinsengOnce*™ **costs less than 60 cents a day** at the Suggested Retail Price of \$17.95 per 30 Tablet Carton.

XETAPHARM™

100 Jersey Avenue
Building B, Ste. 310
New Brunswick, NJ 08901-3279
Tel: (908) 249-0133
Fax: (908) 247-4090

To place an order for *GinsengOnce*™, *GarlicOnce*™
and *GinkgoOnce*™, call customer service at:

1-800-858-5854



A Typical High Performance Liquid Chromatogram of GinsengOnce™

Peng, Chang-Fu, Li, Yu-Jie, et al, Effects of Ginsenosides on Vasodilator Nerve Actions in the Rat Perfused Mesentery are Mediated by Nitric Oxide . J. Pharm Pharmacol., Vol. 47; Pages 614-617, 1995.

Effects of Ginsenosides on Vasodilator Nerve Actions in the Rat Perfused Mesentery are Mediated by Nitric Oxide

CHANG-FU PENG, YU-JIE LI, YUAN-JIAN LI AND HAN-WU DENG

Department of Pharmacology, Hunan Medical University, Changsha, Hunan 410078, P.R. China

Abstract

This study was designed to explore the effect of ginsenosides, saponins from *Panax ginseng*, on the vasodilator nerve actions in the rat perfused mesentery and the mechanism of this effect.

In the rat perfused mesentery, when adrenergic nerves were blocked by guanethidine (5×10^{-6} M) and vascular muscle tone was increased with methoxamine (5×10^{-6} – 10^{-5} M), transmural field stimulation produced a frequency-dependent vasodilator response, which is due to the release of calcitonin gene-related peptide; ginsenosides significantly suppressed this vasodilator response in a concentration-dependent manner (3 – $30 \mu\text{g mL}^{-1}$). After pretreatment with saponin ($50 \mu\text{g mL}^{-1}$, 3 min) to damage endothelial cells, this suppressing effect of ginsenosides was unaltered. However, the effect was abolished by *N*^ω-nitro-L-arginine methyl ester (L-NAME) (10^{-4} M), an inhibitor of nitric oxide synthesis and addition of L-arginine (3×10^{-4} M) restored this suppressing effect. Methylene blue (10^{-5} M), an inhibitor of guanylate cyclase, also abolished the suppressing effect of ginsenosides. However, ginsenosides did not alter the relaxation responses caused by exogenous calcitonin gene-related peptide administration.

We conclude that ginsenosides can produce an inhibitory effect on the vasodilator response prejunctionally in the rat perfused mesentery and that this effect of ginsenosides may be mediated by nitric oxide released from non-adrenergic, non-cholinergic nerves.

Ginsenosides, saponins from *Panax ginseng*, have been documented to possess complex cardiovascular effects. Ginsenosides have been shown to produce different responses in different blood vessels (Chen et al 1984). Recently, it has been reported that vasodilator responses to ginsenosides are related to an L-arginine-nitric oxide-cyclic GMP (L-arg-NO-cGMP) pathway (Chen et al 1993; Kim et al 1993). Besides possessing cardiovascular effects, ginsenosides can modulate the release of neurotransmitter from sympathetic nerves in pithed rats (Zhang & Chen 1987).

It has been demonstrated that capsaicin-sensitive sensory nerves are present in the rat mesentery and that calcitonin gene-related peptide (CGRP), a principal transmitter in vascular sensory nerves, may play a role in the modulation of the total peripheral resistance of the systemic circulation through local reflex mechanisms (Kawasaki et al 1988; Li & Duckles 1992). Our previous study has shown that CGRP release caused by transmural field stimulation can be modulated, in an inhibitory fashion, by the endogenous nitric oxide, probably released by non-adrenergic, non-cholinergic (NANC) nerves (Li et al 1993).

Since mesenteric vasculature is innervated by both sympathetic and capsaicin-sensitive sensory nerves (Kawasaki et al 1988; Li & Duckles 1992), and ginsenosides can inhibit sympathetic neurotransmission (Zhang & Chen 1987), in the present study we examined whether ginsenosides can affect the actions of sensory nerves in the rat perfused mesentery. The modulation of capsaicin-sensitive sensory nerves involves nitric oxide, possibly from NANC nerves (Li et al 1993), and ginsenosides can relax vascular smooth muscle

via an L-arg-NO-cGMP pathway (Chen et al 1993; Kim et al 1993); therefore, we further explored whether the effect of ginsenosides on sensory nerve action is correlated with an L-arg-NO-cGMP pathway.

Materials and Methods

Tissue preparation and perfusion

Mesenteric vasculature of male Sprague-Dawley rats, 180–250 g, was isolated and prepared for perfusion as described previously (Li et al 1993). Rats were decapitated, and the mesenteric artery was quickly cannulated at its origin at the aorta with PE 50 tubing and perfused with Krebs solution, saturated with 95% O₂–5% CO₂. Preparations were then placed in a water-jacketed organ bath (volume 200 mL) maintained at 37°C. The system was perfused with Krebs solution with the help of a peristaltic pump at a rate of $5 \pm 0.2 \text{ mL min}^{-1}$, and superfused by gravity feed at a rate of $1 \pm 0.2 \text{ mL min}^{-1}$. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.8, CaCl₂ 2.5, KH₂PO₄ 1.2, NaHCO₃ 2.5, MgSO₄ 1.2, EDTA.Na 0.107, dextrose 11.5. The perfusion pressure was monitored and recorded by a pressure transducer and a LMS-2B two-channel recorder.

Two platinum electrodes, one placed around the superior mesentery artery and the other resting on the vasculature in a lower part of the preparation, were used to create transmural field stimulation. Transmural field stimulation (amplitude of 60 V, 80 pulses and 3 ms duration) was applied at various frequencies using a Nihonkohden S3201 stimulator. Sufficient time was allowed between each stimulation train for the perfusion pressure to return to a stable level, usually 10–20 min.

Experimental protocols

Tissues were equilibrated for 60 min before each experiment was begun. Responses to transmural field stimulation were stable in the absence of drug. All drugs were administered intraluminally by switching the perfusion solution to a solution containing the drug in the concentrations indicated. For measurement of vasodilator responses, tissues were pretreated with guanethidine (5×10^{-6} M) and then contracted with methoxamine (5×10^{-6} – 10^{-5} M). For ginsenosides, preparations were exposed for 10 min and these remained in the perfusate for the remainder of the study. Transmural field stimulation was applied 10 min after drug administration. For measurement of vasodilator responses to ginsenosides, various concentrations of ginsenosides were tested in a cumulative fashion. In the case of studies of the effect of L-NAME, L-NAME in the presence of L-arginine, or methylene blue on responses to ginsenosides, exposure to L-NAME, L-NAME in the presence of L-arginine, or methylene blue was for 10 min and then responses to transmural field stimulation were tested. After measurement of control responses to transmural field stimulation, tissues were exposed to ginsenosides in the presence of L-NAME, L-arginine and L-NAME, or methylene blue before final stimulation was tested. For CGRP, various concentrations of CGRP were tested. To remove the endothelium, preparations were perfused with distilled water containing saponin ($50 \mu\text{g mL}^{-1}$) for 3 min. Selective removal of the endothelium was confirmed by demonstrating the lack of a vasodilator response to acetylcholine. For all studies a paired design was used, that is, the same tissue was studied both before and after treatment with the particular test reagent.

Drugs

The following drugs were used: saponin, methoxamine, L-arginine, *N*^ω-nitro-L-arginine methyl ester (L-NAME) and guanethidine (Sigma, St Louis, MO); calcitonin gene-related peptide (Peninsula Laboratory, Belmont, CA); ginsenosides (extracted from *Panax ginseng* C. A. Meyer according to the Shibata methods) (Shibata et al 1965) and methylene blue (Medical and Pharmaceutical Co. of China, Beijing). All drugs were dissolved in Krebs solution.

Statistics

Results are expressed as means \pm s.e.m. Student's *t*-test was used to determine statistical differences between two means. The level of significance was chosen as $P < 0.05$.

Table 1. Effect of various concentrations of ginsenosides on vasodilator response to transmural field stimulation ($n = 5$). Relaxation was calculated as percentage of contraction to methoxamine.

Ginsenosides ($\mu\text{g mL}^{-1}$)	Frequency (Hz)		
	1	2	4
0 (Control)	21.1 \pm 7.9	35.7 \pm 9.9	45.1 \pm 5.9
3	18.8 \pm 7.9	27.5 \pm 7.1	38.6 \pm 4.9
10	10.5 \pm 3.7*	19.4 \pm 7.8**	28.2 \pm 8.2**
30	5.6 \pm 3.1**	13.4 \pm 6.1**	22.2 \pm 4.5**

* $P < 0.05$, ** $P < 0.01$ compared with control.

Table 2. Effect of ginsenosides ($10 \mu\text{g mL}^{-1}$) on vasodilator responses to transmural field stimulation in the rat perfused mesentery with endothelium and without endothelium ($n = 5$). Relaxation was calculated as percentage of contraction to methoxamine.

	Frequency (Hz)		
	1	2	4
Control	27.1 \pm 6.8	39.8 \pm 5.4	46.7 \pm 5.3
With endothelium	13.0 \pm 3.2*	25.4 \pm 4.7**	32.0 \pm 7.1*
Control	24.8 \pm 6.0	38.0 \pm 3.4	48.8 \pm 8.8
Without endothelium	17.0 \pm 6.1*	30.0 \pm 2.5**	37.6 \pm 6.8**

* $P < 0.05$, ** $P < 0.01$ compared with control, * $P > 0.20$ compared with corresponding experiment with endothelium.

Results

Guanethidine (5×10^{-6} M) was used to block sympathetic nerves and methoxamine (5×10^{-6} – 10^{-5} M) was added to increase smooth muscle tone in the perfused rat mesenteric vascular bed, with a basal perfusion pressure of 22 ± 3 mmHg ($n = 24$). Transmural field stimulation caused a frequency-dependent vasodilator response, which is due to the release of CGRP (Kawasaki et al 1988). Ginsenosides significantly suppressed vasodilator responses to transmural field stimulation at all indicated stimulation frequencies (Table 1) in a concentration-dependent manner.

To further investigate whether endothelium is involved in the suppressing effect of ginsenosides, tissues were perfused with saponin to damage endothelial cells. As we have shown previously (Li et al 1993), after removal of endothelium by saponin, vasodilator responses to acetylcholine were abolished, and microscopy revealed that the great majority of endothelial cells were removed from the basal lamina. However, removal of endothelium did not alter the suppressing effect of ginsenosides on the vasodilator responses to transmural field stimulation. As shown in Table 2, there was no significant difference in relaxation when endothelium-intact and endothelium-denuded preparations were compared ($P > 0.20$).

The effects of ginsenosides at various concentrations were tested in the presence of guanethidine and methoxamine. At the concentrations of 3 and $10 \mu\text{g mL}^{-1}$, ginsenoside treatment alone did not cause vascular relaxation, while at a higher concentration ($30 \mu\text{g mL}^{-1}$), ginsenosides caused a vasodilator response ($19.9 \pm 4.8\%$, $n = 5$). However, after removal of endothelium, ginsenosides at various concentrations did not cause relaxation of methoxamine-contracted mesentery.

Table 3. Effect of ginsenosides on vasodilator responses to exogenous CGRP ($n = 4$). Relaxation was calculated as percentage of contraction to methoxamine.

	CGRP concn (M)		
	10^{-9}	3×10^{-9}	10^{-8}
CGRP	26.0 \pm 1.1	42.8 \pm 2.4	58.1 \pm 3.8
CGRP + ginsenosides	26.5 \pm 1.1	47.6 \pm 2.7	60.8 \pm 2.8

Table 4. Effect of L-NAME (10^{-4} M) or L-arginine (3×10^{-4} M) in the presence of L-NAME on inhibition by ginsenosides ($10 \mu\text{g mL}^{-1}$) of vasodilator responses to transmural field stimulation ($n = 5$). Relaxation was calculated as percentage of contraction to methoxamine.

	Frequency (Hz)		
	1	2	4
Control	18.1 ± 2.8	32.4 ± 4.2	46.4 ± 6.2
L-NAME + ginsenosides	18.2 ± 2.0	28.8 ± 2.4	45.2 ± 2.1
Control	27.1 ± 9.0	40.6 ± 2.6	52.2 ± 2.4
L-NAME + ginsenosides + L-arginine	$14.9 \pm 3.7^*$	$20.8 \pm 2.5^{**}$	$35.1 \pm 4.6^{**}$

* $P < 0.05$, ** $P < 0.01$ compared with control.

Table 5. Effect of methylene blue (10^{-5} M) on inhibition by ginsenosides ($10 \mu\text{g mL}^{-1}$) of vasodilator responses to transmural field stimulation ($n = 5$). Relaxation was calculated as percentage of contraction to methoxamine.

	Frequency (Hz)		
	1	2	4
Control	25.6 ± 2.8	35.7 ± 3.1	49.6 ± 2.8
Ginsenosides	$14.6 \pm 3.8^*$	$21.1 \pm 2.8^{**}$	$31.2 \pm 1.7^{**}$
Ginsenosides + methylene blue	24.3 ± 4.2	34.8 ± 5.5	47.0 ± 5.8

* $P < 0.05$, ** $P < 0.01$ compared with control.

In tissues pretreated with guanethidine and methoxamine vasodilator responses to exogenous CGRP were unaltered in the presence of $10 \mu\text{g mL}^{-1}$ ginsenosides (Table 3).

The inhibitory action of ginsenoside ($10 \mu\text{g mL}^{-1}$) was completely abolished in the presence of L-NAME (3×10^{-4} M). However, in the presence of L-arginine, a precursor of nitric oxide synthesis, subsequent reversal of the action of L-NAME was observed, as reappearance of inhibitory effects of vasodilator responses to transmural field stimulation (Table 4).

Inhibition of vasodilator responses to transmural field stimulation by ginsenosides was abolished in the presence of 10^{-5} M methylene blue (Table 5).

Discussion

Ginsenosides can inhibit sympathetic nervous activity by an action on presynaptic α_2 -receptors to reduce transmitter release (Zhang & Chen 1987). In the present study, when sympathetic nerve action was blocked by guanethidine, and the perfusion pressure was increased by methoxamine, transmural nerve stimulation caused a frequency-dependent relaxation of the perfused rat mesentery, which is due to the release of CGRP (Kawasaki et al 1988) from the capsaicin-sensitive sensory nerves. Ginsenosides, however, significantly inhibited this frequency-dependent relaxation in a concentration-dependent manner (Table 1). This suggests that ginsenosides cannot only inhibit sympathetic nerve action, but also suppress capsaicin-sensitive sensory nerve action. Furthermore, ginsenosides had no effect on

vasodilator responses to exogenous CGRP. This suggests that inhibition of neurogenic responses by ginsenosides must result from an action at prejunctional sites.

Endothelial factors such as endothelium-derived relaxing factor, besides exerting direct actions on vascular smooth muscle, regulate vascular tone through modulation of sympathetic nerve transmission (Li & Duckles 1992). In the present study, ginsenoside treatment ($30 \mu\text{g mL}^{-1}$) alone caused an endothelium-dependent relaxation. Similar results have been seen in a variety of tissues (Chen et al 1993; Kim et al 1993). These results indicate that ginsenosides can facilitate release of nitric oxide from endothelial cells. However, as has been shown previously, endothelium, which can release nitric oxide, does not mediate the vasodilator effects of sensory nerves, and vasodilator responses to CGRP are endothelium-independent in the rat mesentery (Li & Duckles 1992). Then the inhibitory effect of ginsenosides on sensory nerves in the rat perfused mesentery is mediated by endogenous nitric oxide release from tissues other than the endothelium. In our experiment, in the rat perfused mesenteric vasculature without endothelium, ginsenosides still inhibited vasodilator responses to transmural field stimulation. These results suggest that the inhibitory effect of ginsenosides on vasodilator nerves is not related to endothelial function (Table 2).

Although the endothelium is not involved in modulation of sensory vasodilator nerves in the perfused mesentery of the rat, nitric oxide released from tissues other than the endothelium has been shown to regulate peptidergic neurotransmission in guinea-pig ileum and in the rat perfused mesentery (see Li et al 1993; Gustafsson et al 1990). To explore the possible contribution of endogenous nitric oxide, we used L-NAME, an inhibitor of nitric oxide synthesis. Treatment with L-NAME abolished the inhibition of vasodilator responses to transmural field stimulation by ginsenosides, while this effect of L-NAME was reversed completely by addition of L-arginine. These results suggest that ginsenosides inhibit the actions of sensory vasodilator nerves in the perfused mesentery via stimulation of endogenous nitric oxide release. Our previous investigation has shown that, in the preparations without endothelium, inhibition of synthesis or action of nitric oxide significantly enhances vasodilator responses to transmural field stimulation, and that stimulation of nitric oxide release suppresses vasodilator responses to transmural field stimulation (Li et al 1993).

There is an increasing amount of evidence to suggest that nitric oxide, besides being present in the central nervous system and endothelial cells, may be released from vascular smooth muscle cells as well as from NANC nerves (Garthwaite 1991; Yoshida et al 1993). Recently, it has been reported that the neurally-induced relaxation is associated with nitric oxide released from NANC nerves, that activate guanylate cyclase and increases the synthesis of cGMP in monkey mesenteric artery and in dog and monkey cerebral arteries (Toda & Okamura 1990, 1992). These results, together with the findings of our present study, suggest that it is possible that the inhibitory effect of ginsenosides on the actions of sensory nerves was mediated by the nitric oxide release from NANC nerves in the perfused mesentery of the rat.

Nitric oxide activates guanylate cyclase, with a subsequent elevation of tissue levels of cGMP, resulting in relaxation of vascular smooth muscle and inhibition of neurotransmission (Garthwaite 1991). Methylene blue, an inhibitor of guanylate cyclase, is widely used as a tool to evaluate the mechanism of action of vasodilators (Martin et al 1985; Watanabe et al 1988). We explored the effect of methylene blue on the inhibition of the actions of sensory nerves by ginsenosides. Our results showed that the inhibition of vasodilator responses to transmural field stimulation by ginsenosides was abolished by methylene blue. This suggests the effects of ginsenosides may be secondary to the elevation of cGMP via nitric oxide stimulation of guanylate cyclase.

In summary, the present results suggest that ginsenosides inhibit the actions of vasodilator nerves prejunctionally in the rat perfused mesentery, the inhibitory effect of ginsenosides is mediated by nitric oxide and the nitric oxide which participates in modulating neurotransmission may be from NANC nerves.

Acknowledgement

This work was supported by a Grant from the State Education Commission, P.R. China.

References

- Chen, X., Gillis, C.-N., Moalli, R. (1984) Vascular effects of ginsenosides in vitro. *Br. J. Pharmacol.* 82: 485-491
- Chen, X., Chen, F. Y., Lee, J. J. F. (1993) Ginsenosides induce a NO-mediated relaxation of the corpus cavernosum of the rabbit. *FASEB J.* 7: A770
- Garthwaite, J. (1991) Glutamate, nitric oxide and cell-cell signalling in nervous system. *Trends Neurosci.* 14: 60-67
- Gustafsson, L. E., Wiklund, C. U., Wiklund, N. P., Persson, M. G., Moncada, S. (1990) Modulation of autonomic neuroeffect transmission by nitric oxide in guinea pig ileum. *Biochem. Biophys. Res. Commun.* 173: 106-110
- Kawasaki, H., Takasaki, K., Saito, A., Goto, K. (1988) Calcitonin gene-related peptide acts as a novel vasodilator neurotransmitter in mesenteric resistance vessel of the rat. *Nature* 335: 164-167
- Kim, N. D., Kang, S. Y., Han, J. S. (1993) Effects of ginseng saponin on endothelium-dependent vascular relaxation and cyclic GMP accumulation in the rat aorta. *FASEB J.* 7: A775
- Li, Y. J., Duckles, S. P. (1992) Effect of endothelium on the actions of sympathetic and sensory nerves in the perfused rat mesentery. *Eur. J. Pharmacol.* 210: 23-30
- Li, Y. J., Yu, X. J., Deng, H. W. (1993) Nitric oxide modulates responses to sensory nerve activation of the perfused rat mesentery. *Eur. J. Pharmacol.* 239: 127-132
- Martin, W., Villani, G. M., Jothianandan, D., Furchgott, R. F. (1985) Selective blockade of endothelium-dependent and glycerol trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J. Pharmacol. Exp. Ther.* 232: 708-716
- Shibata, S., Tanaka, O., Soma, K., Iida, Y., Ando, T., Nakamura, H. (1965) Studies in saponins and saponins of ginseng. The structure of panoxachol. *Tetrahedron Lett.* 3: 207-213
- Toda, N., Okamura, T. (1990) Mechanism underlying the response to vasodilator nerve stimulation in isolated dog and monkey cerebral arteries. *Am. J. Physiol.* 259: H1511-H1517
- Toda, N., Okamura, T. (1992) Mechanism of neurally induced monkey mesenteric artery relaxation and contraction. *Hypertension* 19: 161-166
- Watanabe, M., Rosenblum, W. I., Nelson, G. H. (1988) In vivo effect of methylene blue on endothelium-dependent and endothelium-independent dilations of brain microvessels in mice. *Circ. Res.* 62: 86-90
- Yoshida, K., Okamura, T., Snyder, S. H., Toda, N. (1993) Nitric oxide synthase-immunoreactive nerve fibers in dog cerebral and peripheral arteries. *Brain Res.* 629: 67-72
- Zhang, F. L., Chen, X. (1987) Effects of ginsenosides on sympathetic neurotransmitter release in pithed rats. *Acta. Pharmacol. Sin.* 8: 217-220

Mazza, T.S.C. Li. G., Cottrell, A. C., and Gao, L, Ginsenosides in Roots and Leaves of American Ginseng[†], J. Agric. Food Chem., Vol. 44; No. 3, Pages 717-720, 1996.

Ginsenosides in Roots and Leaves of American Ginseng[†]

T. S. C. Li, G. Mazza,* A. C. Cottrell, and L. Gao

Agriculture and Agri-Food Canada, Research Centre, Summerland, British Columbia, Canada V0H 1Z0

The six major ginsenosides, Rg₁, Re, Rb₁, Rc, Rb₂, and Rd, in roots and leaves of American ginseng have been isolated and quantified by high-performance liquid chromatography. In 4-year-old roots, the main ginsenosides were Re and Rb₁, and together they accounted for >75% of the total ginsenosides. In leaves, the concentration and composition of ginsenosides varied with the maturity of the leaf tissue. One-month-old leaves contained 1.33–2.64 g ginsenoside/100 g dry weight, and the ginsenoside Re accounted for >50% of the total concentration. In mature, 4-month-old leaves, the total ginsenoside content ranged from 4.14 to 5.58 g/100 g dry weight, and the ginsenosides Re and Rd each accounted for ~40% of the total ginsenosides. The production site of ginseng influenced the ginsenoside contents of roots and leaves. However, few significant correlations were found between root and leaf ginsenosides and between ginsenoside levels and mineral composition of the leaves and soil.

Keywords: *Panax quinquefolium*; saponins; ginsenosides; HPLC analysis; soil fertility; leaf tissue nutrient status

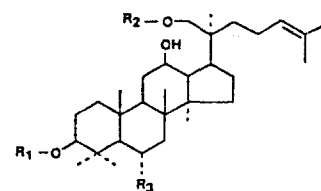
INTRODUCTION

American ginseng (*Panax quinquefolium* L.) is a perennial aromatic herb of eastern North America. It has a long, fleshy root, the shape of which somewhat resembles the human body. Native North Americans used ginseng root as part of their traditional medicine and as an aphrodisiac. Trade in American ginseng began at the beginning of the 1700s, when it was discovered that American ginseng roots possessed properties similar to those of the ginseng from China (*Panax ginseng* C. A. Meyer).

Until quite recently, American ginseng was dug from the wild. As native stands declined, commercial production started in Quebec and later in Wisconsin and Ontario. In British Columbia, commercial ginseng production began in the early 1980s. Presently, >2300 ha of American ginseng, valued at ~\$250 000/ha, are in production in Canada.

The active constituents of ginseng are dammarane saponins, commonly referred to as ginsenosides (Shibata et al., 1985; Tanaka, 1994). The most abundant ginsenosides present in American ginseng are Rb₁, Rb₂, Rc, and Rd, which possess 20(S)-protopanaxadiol as an aglycon; and ginsenosides Rg₁ and Re, which possess 20(S)-protopanaxatriol as an aglycon (Figure 1). Several pharmacological properties have been reported for ginsenosides or ginseng, including effects on the central nervous system, tranquilizing and antipsychotic actions, protection from stress ulcers, increased gastrointestinal mobility, antifatigue action, endocrinological effects, enhancement of sex behavior, and acceleration of both metabolism and synthesis of carbohydrates, lipids, and proteins (Tanaka, 1994).

Quantitative differences in total and individual ginsenosides vary depending on the species, such as *P. ginseng* and *P. quinquefolium* (Lewis, 1988); growing



	R ₁	R ₂	R ₃
R _{g1}	-glc(2→1)glc	-glc(6→1)glc	-H
R _{b2}	-glc(2→1)glc	-glc(6→1)ara(p)	-H
R _c	-glc(2→1)glc	-glc(6→1)ara(f)	-H
R _d	-glc(2→1)glc	-glc	-H
R _e	-H	-glc	-O-glc(2→1)rha
R _f	-H	-H	-O-glc(2→1)glc
R _g	-H	-glc	-O-glc

glc : glucose ara(p) : α-L-arabinopyranose ara(f) : α-L-arabinofuranose rha : rhamnose

Figure 1. Structures of ginsenosides in ginseng.

environment, such as wild and cultivated (Betz et al., 1984); soil and fertility conditions (Konsler et al., 1990); age of the roots (Soldati and Tanaka, 1984); different parts of the plant (Soldati and Sticher, 1980); and extraction methods (Schulten and Soldati, 1981). Quantitative high-performance liquid chromatography (HPLC) analyses of ginsenosides have been reported by several authors (Soldati and Sticher, 1980; Pietta et al., 1986; Kanazawa et al., 1987). However, almost all of these studies have focused on the analyses of ginsenosides in *P. ginseng*, and no systematic analysis of ginsenosides in American ginseng has been reported.

The aim of the present investigation was to study the variation of ginsenosides in roots and leaves of American ginseng grown in three different regions of British Columbia, Canada, and to examine the effects of soil fertility and leaf tissue nutrient status on ginsenoside levels in roots and leaves.

MATERIALS AND METHODS

Roots and leaves of 4-year-old American ginseng (*P. quinquefolium* L.) and soil samples were collected in the summer of 1994 from each of nine commercial ginseng fields in British Columbia. The samples consisted of leaves chosen randomly in the middle of June from six plants per sampling site, and samples of leaves, roots, and soil taken in early September.

* Author to whom correspondence should be addressed [telephone (604) 494-7711; fax (604) 494-0755; internet mazzag@em.agr.ca].

[†] Contribution 940 from the Summerland Research Centre.

Extraction, Purification, and Analysis of Ginsenosides. All the leaf and root samples were refrigerated and then freeze-dried at -65°C within 3 days of sampling. Freeze-dried tissue was ground in a Wiley mill and stored at -35°C until analyzed. Each ground, freeze-dried sample (200 mg) was weighed into a PTFE-stoppered tube and sonicated with 5 mL of 30% (v/v) methanol/water for 40 min in a Bransonic ultrasonic bath. The mixture was centrifuged, and the supernate was filtered through a $0.45\text{-}\mu\text{m}$ hydrophilic Durapore membrane filter (Millipore Corp., Bedford, MA). The crude extract was taken to dryness under a stream of nitrogen, redissolved in 10 mL of water, and refiltered. Two and a half milliliters of the aqueous extract was applied to a C_{18} solid-phase extraction cartridge (Waters Chromatography C_{18} Sep-Pak Classic, 360 mg, preconditioned with 2 mL of methanol and 5 mL of water), and the cartridge was washed with 5 mL of 30% (v/v) methanol. Ginsenosides were eluted with 5 mL of methanol into a NH_2 solid-phase extraction cartridge (Waters Chromatography Sep-Pak Vac NH_2 , 500 mg, preconditioned with 6 mL of water and 6 mL of methanol). The cartridges were further washed with 1 mL of methanol. The eluate, which contained purified ginsenosides, was taken to dryness under a stream of nitrogen. The dried extract was redissolved by sonication and vortexing in 1.0 mL of 25% (v/v) acetonitrile/water, filtered through a $0.45\text{-}\mu\text{m}$ membrane filter, and immediately analyzed for ginsenoside content by HPLC.

Ginsenosides Rb_1 , Rb_2 , Rc , Rd , Re , and Rg_1 were measured on a Hewlett-Packard 1084B HPLC with gradient elution and a reversed-phase (RP) column (Spheri-5 RP-18, $4.6 \times 220\text{ mm}$, $5\text{-}\mu\text{m}$ packing, with RP-18 guard column, $4.6 \times 30\text{ mm}$; Brownlee Labs, Santa Clara, CA). Mobile phases were (A) water and (B) acetonitrile (HPLC grade; J. T. Baker Inc., Phillipsburg, NJ), with a flow rate of 1.5 mL/min and the following gradient: 0–16 min, 21.5% B; 16–50 min, 21.5–40% B; 50–52 min, 40–95% B; 52–55 min, 95% B; 55–60 min, 95–21.5% B; and 60–70 min, 21.5% B. Detection was by UV spectrophotometry at 203 nm, with a reference wavelength of 595 nm. Samples were introduced by autoinjector, with a 20- or 50- μL injection volume.

The concentration of ginsenosides was determined from standard curves prepared by injecting different volumes of stock solutions of authentic ginsenosides Rb_1 , Rb_2 , Rc , Rd , Re , and Rg_1 that were purchased from Carl Roth GMBH (Karlsruhe, Germany).

Leaf and Soil Analyses. Leaf and soil samples were analyzed for nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) in triplicate, according to standard procedures (AOAC, 1980). Soil samples were also assayed for organic matter (OM) and pH. Nitrate N was extracted with 0.25 N HOAc + 0.015 N NH_4F , and its concentration was determined as nitrite by an automated copper-cadmium reduction procedure and color development by sulfanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride. Phosphorus was determined by extraction with 0.25 N HOAc and 0.015 N NH_4F at a 1:10 (v/v) soil:solution ratio, and its concentration was measured on an inductively coupled argon plasma spectrophotometer (ARL 34000). Extractable Ca, Mg, and Na were determined by extraction with a 0.25 N acetic acid and 0.015 N ammonium fluoride solution at a 1:10 (v/v) soil:extractant ratio, and their concentrations were measured on an inductively coupled, argon plasma spectrophotometer.

Statistical Analysis. Statistical data analysis was carried out on a Microvax computer with a GLM procedure, Duncan's New Multiple Range Test, and Pearson's Correlation of SAS (SAS, 1985). Correlation coefficients were calculated between mineral levels in leaf and soil samples and ginsenoside contents of young and mature leaves and roots.

RESULTS AND DISCUSSION

Typical chromatograms of ginseng root, young leaf (l-1), and mature leaf (l-2) extracts and authentic ginsenosides are shown in Figure 2. A highly satisfac-

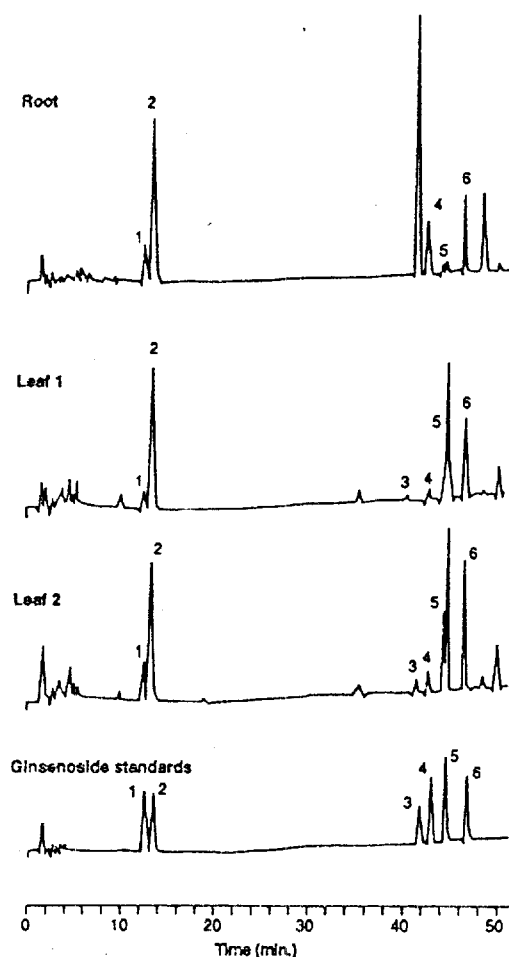


Figure 2. Typical HPLC chromatograms of ginsenosides in American ginseng, monitored at 203 nm: (peak 1) Rg_1 ; (peak 2) Re ; (peak 3) Rb_1 ; (peak 4) Rc ; (peak 5) Rb_2 ; and (peak 6) Rd .

Table 1. Contents (Grams per 100 g of Dry Weight) of Individual and Total Ginsenosides in Young Leaves of American Ginseng Taken from Commercial Fields in British Columbia in 1994

location	leaf (l-1) ginsenosides ^a						total
	Rg_1	Re	Rb_1	Rc	Rb_2	Rd	
1	0.18a	1.34ab	0.04a	0.07bcd	0.10cd	0.18b	1.80bcd
2	0.13a	0.95cd	0.06a	0.05d	0.08d	0.21b	1.43cd
3	0.19a	1.25abc	0.02a	0.06cd	0.14bcd	0.31ab	1.97bcd
4	0.12a	0.77d	0.04a	0.06cd	0.12cd	0.24b	1.33d
5	0.10a	1.04bcd	0.03a	0.06cd	0.05d	0.15b	1.42cd
6	0.12a	1.04bcd	0.05a	0.12abc	0.23abc	0.35ab	1.90bcd
7	0.16a	1.42a	0.04a	0.15a	0.33a	0.55a	2.64a
8	0.17a	0.94cd	0.02a	0.10bcd	0.26ab	0.52a	2.01bc
9	0.14a	1.14abc	0.06a	0.13ab	0.26ab	0.51a	2.23ab
av	0.13	1.10	0.04	0.09	0.20	0.34	1.86

^a Means in each column followed by the same letter are not significantly different ($p \leq 0.05$; $n = 6$).

tory separation of the major ginsenosides of American ginseng including Rg_1 and Re was achieved in <50 min. In agreement with published results (Soldati and Sticher, 1980; Pietta et al., 1986; Kanazawa et al., 1987), the protopanaxtriol derivatives Rg_1 and Re eluted well ahead of the protopanaxadiols Rb_1 , Rc , Rb_2 , and Rd .

In Tables 1, 2, and 3 are the average contents of the total and individual ginsenosides in young leaves (l-1), mature leaves (l-2), and 4-year-old roots from nine commercial fields in the Vernon-Kamloops-Lilloet region

Table 2. Contents (Grams per 100 g of Dry Weight) of Individual and Total Ginsenosides in Mature Leaves of American Ginseng Taken from Commercial Fields in British Columbia in 1994

location	leaf (1-2 ginsenosides) ^a						total
	Rg ₁	Re	Rb ₁	Rc	Rb ₂	Rd	
1	0.35a	1.45a	0.13b	0.22a	0.49cd	1.67a	4.32a
2	0.33a	1.78a	0.22ab	0.39a	0.83ab	2.02a	5.58a
3	0.22a	1.63a	0.26ab	0.28a	0.59bcd	1.43a	4.42a
4	0.24a	1.26a	0.31a	0.33a	0.70abc	2.29a	5.12a
5	0.22a	1.38a	0.22ab	0.36a	0.93a	2.02a	5.12a
6	0.35a	1.76a	0.19ab	0.33a	0.50cd	1.00a	4.14a
7	0.25a	1.71a	0.29ab	0.32a	0.50cd	1.81a	4.38a
8	0.26a	1.54a	0.23ab	0.30a	0.42cd	1.63a	4.38a
9	0.26a	1.53a	0.25ab	0.33a	0.66d	1.75a	4.82a
av	0.28	1.56	0.23	0.32	0.62	1.74	4.15

^a Means in each column followed by the same letter are not significantly different ($p \leq 0.05$; $n = 6$).

Table 3. Contents (Grams per 100 g of Dry Weight) of Individual and Total Ginsenosides in 4-Year-Old Roots of American Ginseng Taken from Commercial Fields in British Columbia in 1994

location	root ginsenoside ^a						total
	Rg ₁	Re	Rb ₁	Rc	Rb ₂	Rd	
1	0.33a	0.87a	0.96bc	0.24a	0.03a	0.26ab	2.69b
2	0.16a	1.35a	0.84c	0.15cd	0.02a	0.27ab	2.79b
3	0.19a	1.03a	0.77c	0.17bcd	0.02a	0.27ab	2.44b
4	0.16a	1.26a	1.46ab	0.17bcd	0.02a	0.23b	3.29ab
5	0.14a	1.23a	1.86a	0.23ab	0.03a	0.40a	3.88a
6	0.19a	1.38a	1.44ab	0.21abc	0.03a	0.31ab	3.56ab
7	0.12a	1.00a	1.09bc	0.17bcd	0.02a	0.25ab	2.65b
8	0.11a	0.84a	1.13bc	0.14d	0.02a	0.29ab	2.52b
9	0.23a	0.96a	1.46ab	0.17bcd	0.02a	0.31ab	3.16ab
av	0.18	1.10	1.22	0.18	0.02	0.29	3.00

^a Means in each column followed by the same letter are not significantly different ($p \leq 0.05$; $n = 6$).

of British Columbia. In young leaves, the content of total and individual ginsenosides, except for Rg₁ and Rb₁, varied significantly from location to location (Table 1). Location 7 had the highest contents of Rb₂, Rc, Rd, Re, and total ginsenosides. In mature leaves, only the ginsenosides Rb₁ and Rb₂ showed significant differences among locations. All other ginsenosides and the total contents were not affected by production site (Table 2). In 4-year-old roots, production site had a statistically significant effect on the content of Rb₁, Rc, Rd, and total ginsenosides (Table 3). Samples from locations 4, 5, 6, and 9 contained the highest levels of total root ginsenosides, and locations 1, 2, 3, 7, and 8 had the lowest levels. On a dry weight basis, mature leaves contained the highest ginsenoside contents, followed by roots and young leaves. Reported contents of ginsenosides in roots of American ginseng range from 1.70 to 2.56% (Soldati and Sticher, 1980; Lewis, 1988; Konsler et al., 1990). Our values are marginally higher than the published levels. This difference could be the result of differences in the age of plants, growing conditions, and soil fertility. In North America, ginseng roots are traditionally harvested after 4 years of cultivation. In this study, ginsenosides were evaluated at this age, although it has been reported that total content of ginsenosides increases with age of the plant (Soldati and Tanaka, 1984) and with moderate addition of lime to the soil (Konsler et al., 1990).

The amount of ginsenosides extracted from leaves varies with the stage of leaf development. As indicated in Tables 1 and 2, the total ginsenosides (Rb₁, Rb₂, Rc, Rd, Re, and Rg₁) of 1.86% for 1-month-old leaves

Table 4. Correlation Coefficients between Ginsenoside Contents of 1-Month-Old and Mature Leaves of American Ginseng

mature leaf	1-month-old leaf						total
	Rg ₁	Re	Rb ₁	Rc	Rb ₂	Rd	
Rg ₁	0.01	0.06	0.55	0.04	-0.06	-0.20	-0.05
Re	0.19	0.33	0.31	0.37	0.39	0.34	0.43
Rb ₁	-0.13	-0.24	-0.11	0.17	0.31	0.39	0.09
Rc	-0.77 ^c	-0.53	0.43	-0.04	-0.10	-0.04	-0.37
Rb ₂	-0.69 ^c	-0.38	0.26	-0.55	-0.68 ^c	-0.59	-0.67 ^c
Rd	-0.33	-0.34	0.09	-0.38	-0.37	-0.27	-0.43
total	-0.02	0.16	-0.43	0.32	0.27	0.23	0.26

^c Significant at the 0.05 level of probability.

increased to 4.16% in mature leaves. These values are within the range of total leaf ginsenosides reported in the literature. Without any indication of specific time of leaf collection, Konsler et al. (1990) reported values of 2.27–2.92% total ginsenosides (A₁, Rb₂, Rd, Re, and Rg₁), whereas Lui and Staba (1980) found a total of 6.6% (Rb₁, Rb₂, Rc, Rd, Re, Rf, Rg₁, and Rg₂).

In our study, significantly different total ginsenosides from nine locations were mainly due to different levels of Rb₁, Rc, and Rd (Table 3), indicating that these three major components are affected by the growing conditions. The Rb₁ in roots from different locations showed the greatest variation from 0.77 to 1.86%. A similar variation was also reported by Sanada and Shoji (1978), with Rb₁ of 1.57%; by Lewis (1988), with Rb₁ of 0.99%; and by Soldati and Sticher (1980), with Rb₁ of 0.26%.

Correlation coefficients relating ginsenoside contents of young and mature leaves are given in Table 4. Significant correlations were obtained between Rb₂ content of mature leaves and Rg₁, Rb₂, and total ginsenoside content of 1-month-old leaves, and between the Rc level of mature leaves and the Rg₁ content of young leaves. The sign of these coefficients suggests that low levels of ginsenosides in young leaves lead to an increased accumulation of ginsenosides in mature leaves. However, the limited number of significant correlation coefficients between the individual and total ginsenoside contents in leaves at the two stages of maturity indicate that factors other than maturity influence the accumulation of ginsenosides in leaves of American ginseng.

Correlations between ginsenoside contents of leaves and roots were very low. The only significant ($p \leq 0.05$) combinations were Rg₁ of young leaves with Rb₁ ($r = -0.75$) and total ($r = -0.89$) of roots; total ginsenosides of young leaves with Rg₁ ($r = -0.83$) and total content ($r = 0.69$) of roots; and Rb₁ and Rc of mature leaves with Rb₂ ($r = -0.76$) and Re ($r = -0.71$) of roots, respectively. The sign of these coefficients indicates that increases in leaf ginsenosides correspond to lower ginsenoside contents in roots. These findings agree with previous studies (Konsler et al., 1990) that have shown that the relationship between leaf and root ginsenosides contents is generally poor.

Correlation analyses between N, P, K, Mg, and Ca contents of 1-month-old leaves and ginsenoside levels of leaves and roots revealed that the majority of the significant correlations were between ginsenosides and N levels of young leaves. The sign of the coefficients was negative for N and Re, Rb₁, and total ginsenosides of young leaves, and Re of mature leaves, and positive for N and Rb₁ and total ginsenosides of roots. There were no significant correlations between P contents and ginsenosides, whereas K, Mg, and Ca were correlated with Re of young leaves and roots, Rb₂ of roots, and Re

of young leaves, respectively. There were no correlations between N, P, K, Mg, and Ca of mature leaves and ginsenosides of leaves at the same stage of maturity. Contents of N, Mg, and Ca in the soil showed significant negative correlation with Rb₂ ($r = -0.77$), Rb₁ ($r = -0.70$) and Rc ($r = -0.70$), and Rb₁ ($r = -0.79$), respectively. The Mg and Ca content of mature leaves showed significant negative correlation with Re ($r = -0.69$) and Rg₁ ($r = -0.77$) of root, whereas only N content in the soil showed positive correlation with Rd ($r = 0.82$) and total ginsenosides of root ($r = 0.67$).

There is almost no published information on the effects of cultural practices and nutrient status of leaves and soil on ginsenosides of American ginseng, thus no comparison of our results with published data can be made. The relatively strong and consistent correlations between N contents and ginsenosides of leaves and roots, however, indicate that N fertilization of ginseng should be carried out cautiously.

Organic matter, pH, P, and K levels of soil were not significantly correlated with ginsenosides of mature leaves and roots, even though Konsler et al. (1990) noted that soil pH and phosphate levels result in significant changes in the tissue content of certain ginsenosides. The level of N in the soil showed a significant negative correlation with Rb₂ of mature leaves and a positive correlation with Rd and total ginsenosides of root, whereas Mg and Ca levels in the soil showed negative correlations only with Rb₁ and Rc of mature leaves and not with ginsenosides in root. These observations are in agreement with Konsler et al. (1990), who concluded that soil fertility factors, including Mg and Ca, are more closely related to leaf than to root ginsenoside concentrations. Further work is required to better elucidate the effects of soil fertility and timing of application of fertilizers on ginsenosides of American ginseng.

ACKNOWLEDGMENT

We thank the participating ginseng producers of British Columbia for their cooperation and France Côté for technical help.

LITERATURE CITED

- AOAC. *Official Methods of Analysis*, 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.
- Betz, J. M.; Der Morderosian, A. H.; Lee, T. M. Continuing studies on the ginsenoside content of commercial ginseng products by TLC and HPLC. II. Proceedings of the 6th North American Ginseng Conference; Guelph, ON, 1984; pp 65-53.
- Kanazawa, H.; Nagata, Y.; Matsushima, Y.; Tomoda, M.; Takai, N. High-performance liquid chromatographic analysis of ginsenosides in *Panax ginseng* extracts using glass-ODS column. *Chromatographia* 1987, 24, 517.
- Konsler, T. R.; Zito, S. W.; Shelton, J. E.; Staba, E. J. Lime and phosphorus effects on American ginseng: II. Root and leaf ginsenoside content and their relationship. *J. Am. Soc. Hortic. Sci.* 1990, 115, 575-580.
- Lewis, W. H. Ginseng: a medical enigma. In *Plants in Indigenous Medicine and Diet Biobehavioral Approaches*; Etkin, N. L., Ed.; Redgrave: New York, NY, 1988.
- Lui, J. H. C.; Staba, E. J. The ginsenosides of various ginseng plants and selected products. *J. Nat. Prod.* 1980, 43, 340-346.
- Pietta, P.; Mauri, P.; Rava, A. Improved high-performance liquid chromatographic method for the analysis of ginsenosides in *Panax ginseng* extracts and products. *J. Chromatogr.* 1986, 356, 212.
- Sanada, S.; Shoji, J. Comparative studies on the saponins of ginseng and related crude drugs. *Shoyakugaku Zasshi* 1978, 32, 96-99.
- SAS. *SAS User's Guide: Statistics*; Statistical Analysis System: Cary, NC, 1985.
- Schulten, H. R.; Soldati, F. Identification of ginsenosides from *Panax ginseng* in fractions obtained by high-performance liquid chromatography by field desorption mass spectrometry, multiple internal reflection infrared spectroscopy and thin-layer chromatography. *J. Chromatogr.* 1981, 212, 37-49.
- Shibata, S.; Tanaka, O.; Shoji, J.; Saito, H. Chemistry and pharmacology of *Panax*. In *Economic and Medicinal Plant Research*; Wagner, H., Hikino, H., Farnsworth, N. R., Eds.; Academic: Tokyo, 1985, Vol. 1, pp 218-284.
- Soldati, F.; Sticher, O. HPLC separation and quantitative determination of ginsenosides from *Panax ginseng*, *Panax quinquefolium* and from ginseng drug preparations. *Planta Med.* 1980, 38, 348-357.
- Soldati, F.; Tanaka, O. *Panax ginseng*: relation between age of plant and content of ginsenosides. *Planta Med.* 1984, 50, 351-352.
- Tanaka, O. Ginseng and its congeners. In *Food Phytochemicals for Cancer Prevention II*; Ho, C.-T., Osawa, T., Huang, M.-T., Rosen, T. R., Eds.; American Chemical Society: Washington, DC, 1994; pp 335-341.

Received for review May 22, 1995. Revised manuscript received September 7, 1995. Accepted November 3, 1995.^{*}

JF950309F

^{*} Abstract published in *Advance ACS Abstracts*, February 1, 1996.

Chen, Xiu, Gillis, C. Norman and Moalli, R., Vascular effects of ginsenosides *in vitro*, Br. J. Pharmac. Vol. 82; Pages 485-491, 1984.

Vascular effects of ginsenosides *in vitro*

Xiu Chen¹, C. Norman Gillis & Richard Moalli

Departments of Anesthesiology and Pharmacology, Yale University School of Medicine, New Haven, CT 06510, U.S.A.

- 1 Ginsenosides (saponins extracted from *Panax ginseng*) elicit qualitatively and quantitatively different responses in isolated, contracted ring preparations of different blood vessels from rabbits, dogs and humans.
- 2 Ginsenosides themselves did not affect the tone of 'resting' isolated blood vessels directly, but contracted slightly the renal vein of rabbits at the maximum concentration tested. The mixture caused relaxation of the noradrenaline (NA) or prostaglandin F_{2α} (PGF_{2α})-induced contraction of the pulmonary artery and intrapulmonary artery of rabbits, and the PGF_{2α}-induced contraction of the canine mesenteric vein.
- 3 Ginsenosides potentiated, in a concentration-dependent manner, the contractile responses of renal veins of dogs and rabbits to PGF_{2α}.
- 4 The reason for such heterogeneous responses of different blood vessels to ginsenosides is unknown. It is suggested that either potentiation of contraction or relaxation of contracted blood vessels might be mediated by interaction with endogenous vasoactive substances. The potentiation of PGF_{2α}-induced contraction may be related to the reduction of renal blood flow observed in anaesthetized dogs. The simultaneous contraction and relaxation effects may explain its biphasic actions on blood pressure.

Introduction

Panax ginseng has been used for more than two thousand years as a general tonic in traditional Chinese medicine. Recently it was reported that ginsenosides, saponins extracted from *Panax ginseng*, provide some protection against experimental myocardial infarction in rabbits (Chen *et al.*, 1980). In order to elucidate the mechanism of this action, the cardiovascular and haemodynamic effects of ginsenosides were studied in experiments in intact dogs. It was found that the mixture significantly reduced vertebral and femoral vascular resistance but increased renal vascular resistance mainly by decreasing renal blood flow (Chen *et al.*, 1982a,b). These findings suggested that ginsenosides may produce different responses in different blood vessels. The purpose of the present study was to examine the validity of this assumption by evaluating the response to ginsenosides of a variety of isolated blood vessels prepared from rabbits, dogs and man.

Methods

Male New Zealand albino rabbits (2.2–2.8 kg) were anaesthetized by intravenous injection of allobarbitone (125 mg kg⁻¹) and urethane (500 mg kg⁻¹) followed by administration of heparin (500 units kg⁻¹). The lungs and heart were removed and placed in Krebs-bicarbonate solution and aerated continuously with 95% O₂ and 5% CO₂. Segments (2–3 cm) of the ascending aorta and right pulmonary artery (RPA) were removed. Right intrapulmonary artery segments were carefully isolated and cannulated with a fine polypropylene tubing (1 mm o.d.) and removed. After opening the abdominal cavity, the right renal artery and vein segments were isolated, cannulated and removed.

Male mongrel dogs (18–23 kg) were anaesthetized with pentobarbitone (30 mg kg⁻¹) followed by heparin (500 units kg⁻¹). Segments of right renal artery and vein and mesenteric vein were carefully isolated and removed.

Human saphenous veins employed were segments of unused tissue removed from patients (60–70 years of age) undergoing coronary bypass surgery.

Only vessels from 2.5 to 6.0 mm diameter were

¹ Present address: Department of Pharmacology, Hunan Medical College, Changsha 410008, People's Republic of China.

used in this study. Each vessel was carefully cleaned of surrounding tissue, cut into cylindrical segments 5–6 mm in length and mounted on fine stainless steel hooks (Hooker *et al.*, 1977; Altieri *et al.*, 1983a). In each experiment four different blood vessels were suspended in 10 ml organ baths containing Krebs-bicarbonate solution at 37°C and continuously aerated with 95% O₂ plus 5% CO₂. Changes in force were measured with semi-isometric force transducers (FT03 for rabbit: pulmonary and renal arteries, load 5g; dog: renal artery, load 7–10g; renal vein 3–6g; mesenteric vein 2–5g; human saphenous vein 8–10g; Statham G7A for rabbit intrapulmonary artery load 5g; G10B for rabbit renal vein load 0.5g) and recorded on a Grass Model 7 polygraph. Preload tension was determined as described previously (Altieri *et al.*, 1983a). Ring preparations were allowed to equilibrate for 2 h under optimal applied load. The bath solution was changed by overflow every 15 min during the equilibration period.

Cumulative ginsenoside concentration-response curves were produced by the addition of solutions of ginsenosides to attain final concentrations of 10 µg to 1000 µg ml⁻¹ in the bathing medium. After repeated washing and equilibration for approximately 60 min, cumulative concentration-response curves were generated with freshly prepared solutions of noradrenaline (NA) and prostaglandin F_{2α} (PGF_{2α}). When preparations were maximally contracted by the agonists (NA, 5 × 10⁻⁶ M; PGF_{2α}, 5 × 10⁻⁵ M), ginsenoside solution was added to the organ baths in a cumulative manner to observe its effect on NA or PGF_{2α}-induced contractile responses.

Drugs

Ginsenosides were extracted from *Panax ginseng* C.A. Meyer according to the Shibata method (Shibata *et al.*, 1982). Ginsenoside, as well as L-

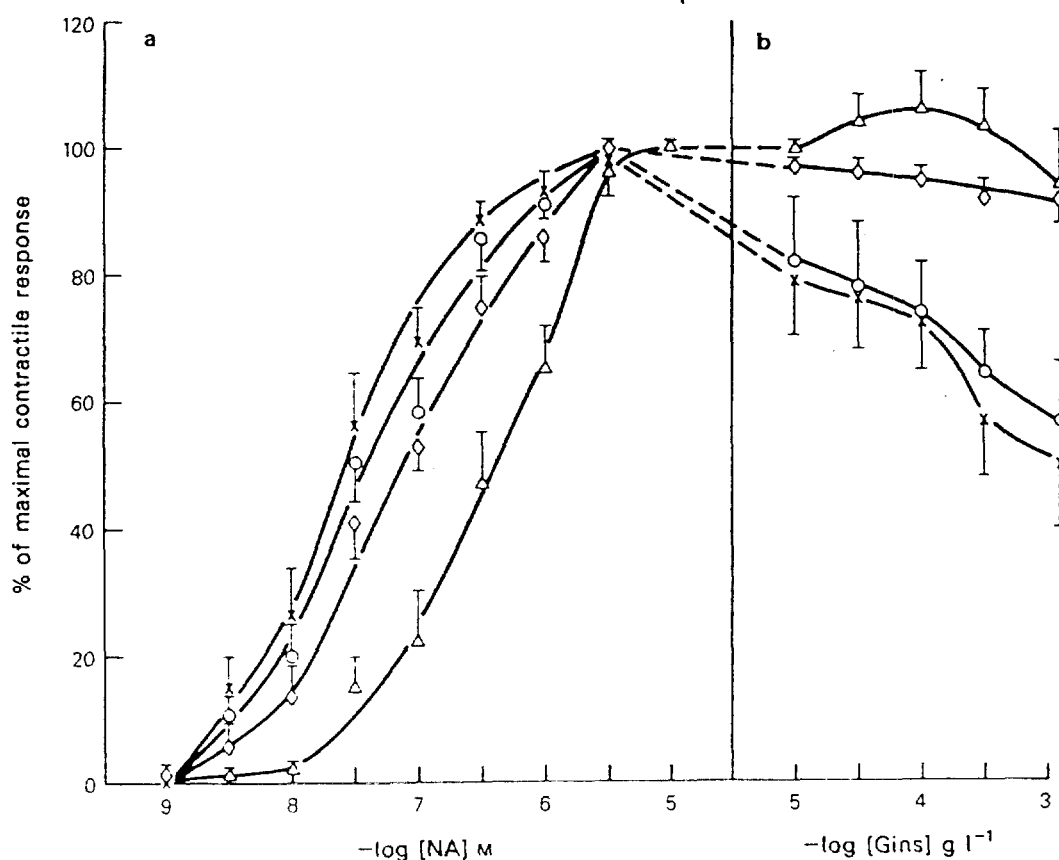


Figure 1 Effect of ginsenosides on noradrenaline (NA)-induced contractile responses of rabbit blood vessels. (a) Cumulative dose-response curves of the intrapulmonary artery (x), pulmonary artery (O), aorta (◇) and renal artery (Δ) to NA. (b) The effect of ginsenosides on the maximal contraction induced by noradrenaline in the different blood vessels. Data points represent mean ± s.e. mean (vertical bars) of 6 vessels (except renal artery, *n* = 5)

noradrenaline bitartrate (NA, Sigma Chemical Co.) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Upjohn, Kalamazoo, MI) were prepared in the Krebs solution immediately before use. All solutions were kept on ice and protected from light during the experiments.

Analysis of data

Concentration-response data were expressed in grams of developed force and normalized as % of the maximum response (either contraction or relaxation) of the vessel in each experiment. The mean and standard error of the mean were calculated for each vessel. Statistical significance was evaluated by the t

test for individual or group comparisons. P values of 0.05 or less were considered to be significant.

Results

Rabbit blood vessels

Addition of ginsenosides (10 – $1,000 \mu\text{g ml}^{-1}$ in the bathing medium) did not contract rabbit pulmonary, intrapulmonary or renal arteries or aorta in the absence of an agonist. The maximal concentration ($1000 \mu\text{g ml}^{-1}$) slightly contracted the renal vein, causing the development of a tension of 55 mg , equivalent to 17% of the maximal contraction induced by

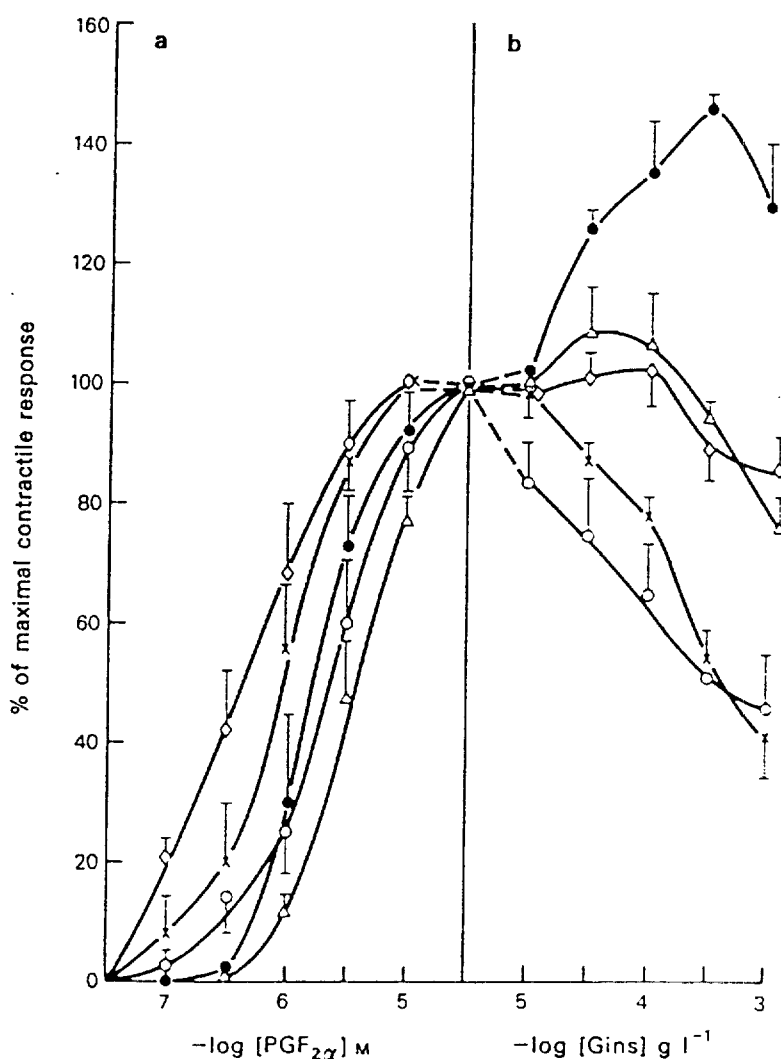


Figure 2 Effect of ginsenosides on the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-induced contractile responses of rabbit blood vessels. (a) Cumulative dose-response curves of pulmonary artery (○), renal vein (●), renal artery (Δ), intrapulmonary artery (×) and aorta (◇) to $PGF_{2\alpha}$. (b) Effects of ginsenosides on the $PGF_{2\alpha}$ -contracted blood vessels. Data points represent mean \pm s.e.mean (vertical bars) of 4–7 vessels.

PGF_{2α}. However, in some blood vessels ginsenosides did affect the contraction induced by either NA or PGF_{2α}. Figure 1a shows the cumulative concentration-response curve to NA of the intrapulmonary and pulmonary artery, and renal artery (from left to right). When the maximal contraction was obtained to 5×10^{-5} M NA and ginsenosides were added to the organ bath in a cumulative manner they significantly relaxed the pulmonary and intrapulmonary arteries in a concentration-dependent manner (from $10 \mu\text{g ml}^{-1}$ to $1000 \mu\text{g ml}^{-1}$). However, ginsenosides only slightly contracted the renal artery and had no apparent effect on the previously contracted aorta (Figure 1). Similar effects were ob-

served when blood vessels were contracted by PGF_{2α}. Thus, when a maximal contraction was induced by PGF_{2α} ginsenosides also relaxed the pulmonary and intrapulmonary arteries in a concentration-dependent manner. However, ginsenosides produced no significant effect on the contracted renal artery or aorta, although at very high concentrations (500 – $1,000 \mu\text{g ml}^{-1}$) these vessels relaxed slightly (Figure 2). A unique effect shown in Figure 2 is that ginsenosides potentiated the contractile response of the renal vein to PGF_{2α}. Thus the rabbit renal vein responds to ginsenosides in a qualitatively different manner from the other arteries studied.

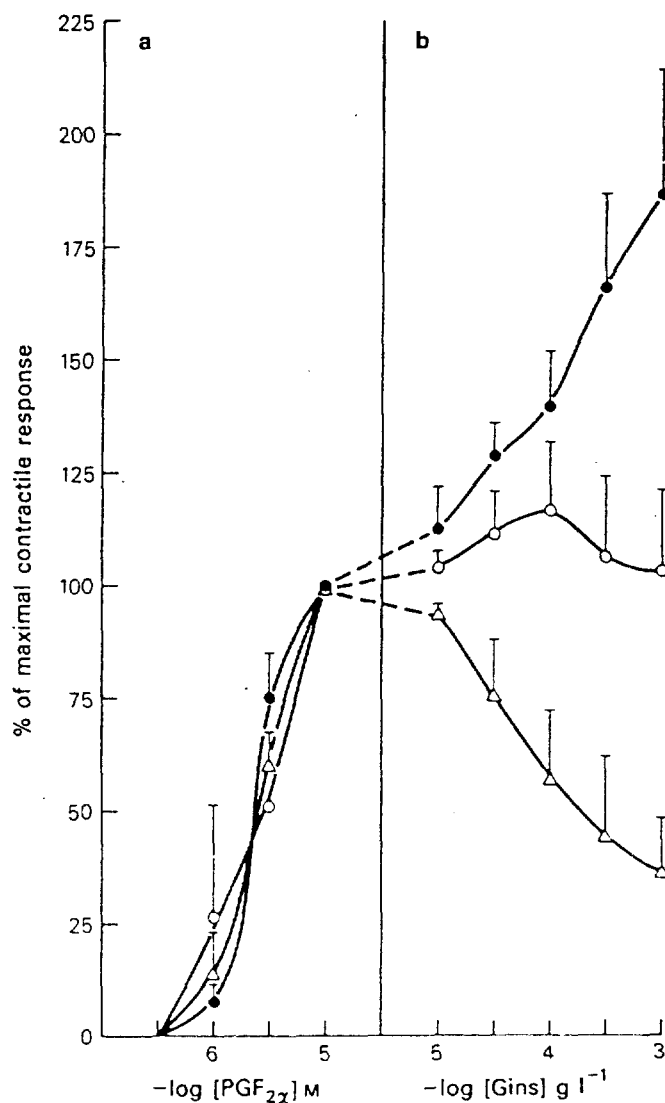


Figure 3 Effect of ginsenosides on prostaglandin F_{2α} (PGF_{2α})-induced contraction of dog blood vessels. (a) The cumulative dose-response curves of renal artery (○), mesenteric vein (Δ) and renal vein (●) to PGF_{2α}. (b) Effects of ginsenosides on these contracted vessels. Data points represent mean \pm s.e. mean (vertical bars) of 3–4 vessels.

Canine blood vessels

Ginsenosides alone did not significantly affect the canine isolated renal artery and vein and mesenteric vein. However, as shown in Figure 3, the mixture also potentiated the contractile response of the canine renal vein to $\text{PGF}_{2\alpha}$, but had no significant effect on the contracted renal artery. In contrast, it markedly relaxed $\text{PGF}_{2\alpha}$ -induced tone in the canine mesenteric vein. Thus, ginsenosides also acted qualitatively differently in the canine renal artery and vein.

Human vessels

As shown in Figure 4a, ginsenosides alone in high concentrations slightly contracted the saphenous vein. This small contraction could also be elicited when the tissue was previously contracted by $\text{PGF}_{2\alpha}$, although no potentiation was seen.

Discussion

In the present study we found that ginsenosides had little direct effect on 'resting' isolated blood vessels. Only at high concentrations did ginsenosides contract the rabbit renal vein. However, the saponin mixture significantly relaxed the contracted (NA or $\text{PGF}_{2\alpha}$ -induced contraction) rabbit pulmonary and intrapulmonary artery, and the contracted ($\text{PGF}_{2\alpha}$ -induced contraction) canine mesenteric vein. A qualitatively different effect was observed with the rabbit or dog renal vein. In these preparations, ginsenosides potentiated the contractile response to $\text{PGF}_{2\alpha}$. This potentiation of the contractile response is recognized as being true potentiation, since ginsenosides alone produced only 17% of the maximum contractile response to $\text{PGF}_{2\alpha}$; however, if added at the peak of the $\text{PGF}_{2\alpha}$ induced contractile response ginsenosides further increased the contraction of the renal vein by an additional 46%; that is, three times the response

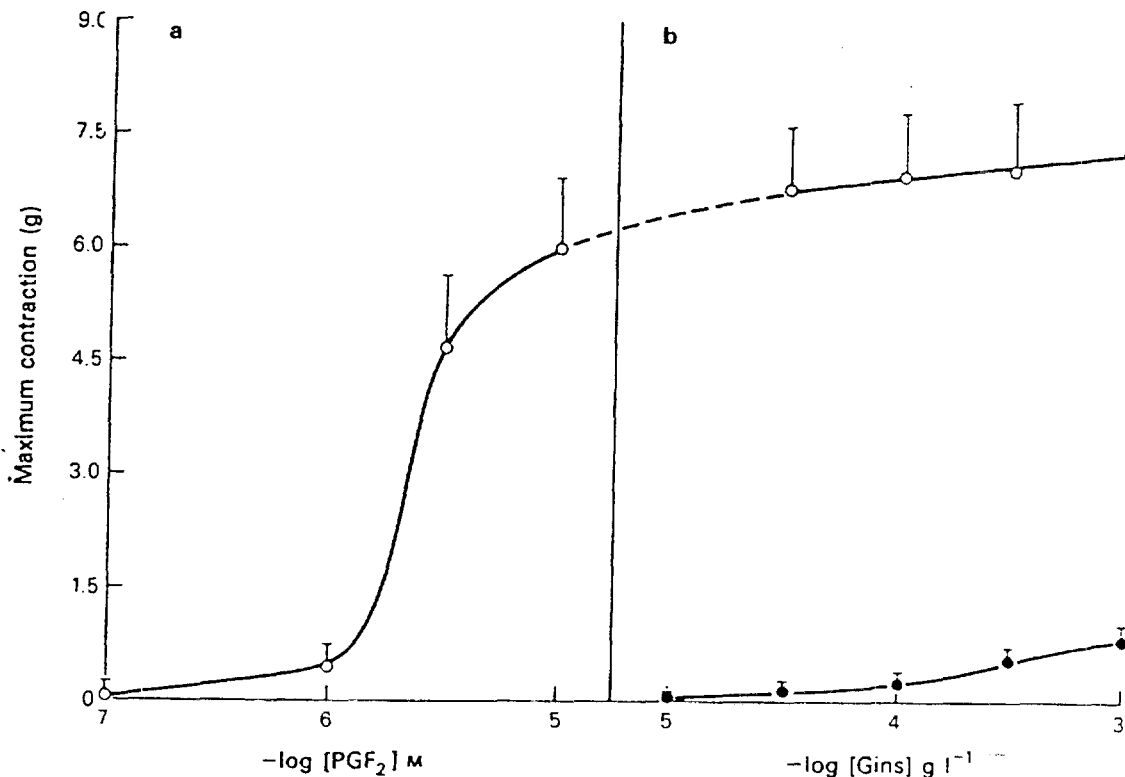


Figure 4 Effect of ginsenosides on human saphenous veins by itself and in combination with prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$). (a) The dose-response curve of human saphenous veins to $\text{PGF}_{2\alpha}$. (b) The effect of ginsenosides on human saphenous vein normally (●) and after the vein had contracted to $\text{PGF}_{2\alpha}$ (○). Data points represent mean \pm s.e. mean (vertical bars) values.

produced by ginsenosides alone.

Considerable evidence has been published indicating that PGF_{2α} can be synthesized in the kidney of rats, rabbits and man (Dusing *et al.*, 1978; Oliw, 1980; Sraer *et al.*, 1982) and released in the canine renal vein (Chandler & Giri, 1981; Lonigro *et al.*, 1982). There is also evidence that prostaglandins play an important role in the regulation of renal blood flow (Terragno *et al.*, 1977). Furthermore, it has been shown that among the prostaglandins PGF_{2α} is the most potent vasoconstrictor of canine isolated renal vein (Chand & Altura, 1981). It is also of interest to note that the pressor activity of PGF_{2α} is considered primarily to result from venoconstriction (Ducharme *et al.*, 1968). Therefore it seems reasonable to speculate that the reduction in renal blood flow induced by ginsenosides observed with the intact dog (Chen *et al.*, 1982a) may result from a potentiation of the renal vasoconstriction caused by endogenous PGF_{2α}.

In recent years the heterogeneity of responses in different blood vessels has been noted. For example, veins from different locations and of different sizes were found to have different innervation patterns and sensitivity to NA. Bevan *et al.* (1974) interpreted these differences as reflecting different functional requirements. Recently we demonstrated that extrapulmonary and intrapulmonary arteries of the rabbit have different responses (Altieri *et al.*, 1983b). Even in the same rabbit ear artery, the density of innervation in the proximal region is about twice that in the distal region, and the former also responded more quickly than the latter (Griffith *et al.*, 1982). However, the qualitative difference we observed in the effects of ginsenosides on different blood vessels has not been described previously. It is

conceivable that the relaxant effect of ginsenosides in the pulmonary and intrapulmonary arteries might be mediated through release of endogenous vasodilator substances. It has been shown, for example, that the endothelial cell of the aorta contains acetylcholine, which can be released by injury (Furchgott & Zawadzki, 1980). Acetylcholine, acting on muscarine receptors, stimulates formation of cyclic GMP which then relaxes arterial smooth muscle (Furchgott & Jothianandan, 1983).

Our data may also explain the complicated and seemingly conflicting haemodynamic effects of ginsenosides described in the literature. For example, *Panax ginseng* has been described as having either hypotensive (rabbits; Hsu, 1955), hypertensive (dogs; Chang, 1959); or biphasic effects on blood pressure of dogs and rats (Chen *et al.*, 1982a,b; Wood *et al.*, 1964). As shown in the present study, ginsenosides can cause both vasoconstriction or vasodilatation in different vessels. Thus, the net effect on blood pressure and other haemodynamic parameters will reflect the predominant vascular site of action. In addition, ginsenosides used in our study are a complex mixture of ginseng saponins, each of which may have different effects (Kaku *et al.*, 1975), thus further complicating prediction of the cardiovascular effect of ginseng.

We gratefully acknowledge the careful technical assistance of Ms Mary Beth Clark and secretarial assistance of Ms Suzanne Abrams. We are very grateful to Dr Graeme L. Hammond, Department of Surgery, Yale University School of Medicine, for making available to us samples of human saphenous vein.

This work was supported by U.S. Public Health Service Grants HL-07410 and HL-13315.

References

- ALTIERE, R.J., DOUGLAS, J.S. & GILLIS, C.N. (1983a). Potassium chloride- and norepinephrine-induced contractile responses in rabbit pulmonary blood vessels. *J. Pharmac. exp. Ther.*, **224**, 572–578.
- ALTIERE, R.J., DOUGLAS, J.S. & GILLIS, C.N. (1983b). Pharmacologic analysis of norepinephrine responses in rabbit pulmonary blood vessels. *J. Pharmac. exp. Ther.*, **224**, 579–589.
- BEVAN, J.A., HOSMER, D.W., LIUNG, B., PEGRAM, B.L. & SU, C. (1974). Innervation pattern and neurogenic response of rabbit veins. *Blood Vessels*, **11**, 172–182.
- CHAND, N. & ALTURA, B.M. (1981). Influence of primary prostaglandins on isolated canine renal arteries and veins. *Prostaglandins & Med.*, **7**, 49–62.
- CHANDLER, D.B. & GIRI, S.N. (1981). Effect of acute paraquat toxicity on circulation levels of arachidonic acid, PGF_{2α} and PGE₁ in the beagle. *Prostaglandins & Med.*, **7**, 571–579.
- CHANG, B.H. (1959). The cardiovascular action of *Panax ginseng*. *Acta physiol sinica*, **23**, 1–8.
- CHEN, X., FANG, Y.X. & TANG, X.L. (1980). The therapeutic effect of ginsenosides extracted from *Panax ginseng* on the experimental myocardial infarction of rabbits in comparison with clonidine and N-methyltyramine. *Bull. of Hunan Medical College*, **5**, 233–236. (See also Chem. Abstr. 95: 126044.)
- CHEN, X., ZHU, Q.Y., LIU, L.Y. & TANG, X.L. (1982a). Effect of ginsenosides on cardiac performance and hemodynamics of dogs. *Acta pharmaco sinica*, **3**, 236–239.
- CHEN, X., DENG, H.W., LIU, L.Y. & FANG, Y.X. (1982b). Experimental study on the cardiovascular effects of ginsenosides. *Chinese J. Cardiol.*, **10**, 147–150.
- DUCHARME, D.W., WEEKS, J. & MONTGOMERY (1968). Studies on the mechanism of the hypotensive effect of prostaglandin F_{2α}. *J. Pharmac. exp. Ther.*, **160**, 1–10.
- DUSING, R., ATTALLAH, A.A., PREZYNA, A.P. & LEE, J.B. (1978). Renal biosynthesis of prostaglandin E₂ and F_{2α}:

- dependence on extracellular potassium. *J. lab. clin. Med.*, **92**, 669-677.
- FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373-376.
- FURCHGOTT, R.F. & JOTHIANANDAN, D. (1983). Relation of cyclic GMP levels to endothelium-dependent relaxation by acetylcholine in rabbit aorta. *Fedn. Proc.*, **42**, 619.
- GRIFFITH, S.G., CROWE, R., LINCOLN, J., HAVEN, A.J. & BURSTOCK, G. (1982). Regional differences in the density of perivascular nerves and varicosities, noradrenalin content and responses to nerve stimulation in the rabbit ear artery. *Blood Vessels*, **19**, 41-52.
- HOOKE, C.S., CALKINS, P.J. & FLEISCH, J.H. (1977). On the measurement of vascular and respiratory smooth muscle responses *in vitro*. *Blood Vessels*, **14**, 1-11.
- HSU, C.T. (1956). On *Panax ginseng* Nies, *Var. notoginseng* Burkill. *Jap. J. Pharmac.*, **6**, 18-28.
- KAKU, T., MIYATA, T., USUNO, T., SAKO, I. & KINOSHITA, A. (1975). Chemico-pharmacological studies on saponin of *ginseng* C.A. Meyer. II. Pharmacological part. *Arzneim Forsch.*, **25**, 539-547.
- LONIGRO, A.J., BRASH, D.W., STEPHENSON, A.H. & HEITMANN, L.J. (1982). Effect of ventilatory rate on renal venous PGE₂ and PGF_{2α} efflux in anesthetized dogs. *Am. J. Physiol.*, **242**, 38-45.
- OLIW, E. (1980). A radioimmunoassay for 6-keto-PGF_{2α} utilizing an antiserum against 6-methoxime-prostaglandin F_{2α}. Application to study renal *in vitro* synthesis of prostacyclin. *Prostaglandins*, **19**, 271-284.
- SHIBATA, S., TANAKA, O., SOMA, K., IIDA, Y., ANDO, T. & NAKAMURA, H. (1965). Studies in saponins and sapogins of ginseng. The structure of panoxachol. *Tetrahedron Letts.*, **3**, 207-213.
- SRAER, J., ARDAILLOU, N., SRAER, I.D. & ARDAILLOU, R. (1982). *In vitro* prostaglandin synthesis by human glomeruli and papillae. *Prostaglandins*, **23**, 855-864.
- TERRAGNO, N.A., TERRAGNO, D.A. & MCGIFF, J.C. (1977). Contribution of prostaglandins to the renal circulation in conscious, anesthetized and laparotomized dogs. *Circ. Res.* **40**: 590-595.
- WOOD, W.B., ROH, B.L. & WHITE, R.P. (1964). Cardiovascular actions of *Panax ginseng* in dogs. *Jap. J. Pharmac.*, **14**, 284-294.

(Received October 28, 1983.)